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Isolation of human genital TRIC agents in nongonococcal urethritis and Reiter's disease

By an irradiated cell culture method

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In an earlier study, Ford and McCandlish (1969) isolated TRIC agents, by the yolk-sac method, from fifteen of 133 urethral scrapings of men with nongonococcal urethritis. Gordon and Quan (1965) had previously found that TRIC agents would propagate in irradiated McCoy cells and could be readily demonstrated by the resulting inclusions which stained purple with iodine because of their glycogen content. Subsequently, Gordon, Harper, Quan, Treharne, Dwyer, and Garland (1969) considered that the McCoy cell method was more sensitive in addition to being quicker and more convenient than the yolk-sac method in isolating TRIC agents from clinical specimens. It therefore seemed advisable to perform an evaluation of the two methods in Vancouver and the following paper describes our results.

Methods

The techniques of Gordon as described in the publications quoted above were followed as closely as possible. Dr. Gordon not only very kindly supplied a stock culture of his McCoy cells which were thereafter propagated in our laboratory, but also instructed one of us (DKF) in the techniques during a visit to Dr. Gordon's laboratory. The only apparent difference between his method and ours was that we used only streptomycin as an antibiotic whereas he combined streptomycin with either ristocetin or vancomycin in his studies; bacterial contamination was not a problem, however, as our study was confined to urethral scrapings from men. The irradiation was provided by cobalt^{60}.

The patients were derived from the same source as our previous study. Men attending the Vancouver V.D. Control Clinic for nongonococcal urethritis were selected for study if the urethral exudate showed no gonococci by stained smear and/or culture, and if definite clinical evidence of urethritis was present. One of us (DKF) personally obtained all the scrapings with a Dunlop-Jones curette and placed them in 1 ml. volumes of 0·2 M sucrose phosphate buffer at pH 7·0. The specimens were frozen at −70°C. until tested. The overwhelming majority of patients were Caucasians. Patients with Reiter's syndrome had active urethritis and had not received broad-spectrum antibiotics.

The egg yolk-sac method was the same as that employed in the previous study.

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Results

The accompanying Table outlines the results obtained during an 18-month study period which was conducted in three stages. An initial survey was made of 45 males with nongonococcal urethritis and urethral scrapings yielded only two TRIC isolates. It was then considered advisable to study a group of cases with the yolk-sac method in conjunction with the McCoy cell method. A further 44 cases were thus evaluated; two cases yielded TRIC agents by both methods and neither method yielded any other isolates. Finally, after further correspondence with Dr. Gordon and a change of technicians, a further 62 patients were studied and four additional isolates were obtained. The overall isolation rate by the McCoy cell method was therefore eight agents from 151 urethral scrapings from patients with uncomplicated nongonococcal urethritis.

TABLE *Isolation of* TRIC *agents from urethral scrapings by the McCoy cell method*

Studies	*No. of cases*	*No. of* TRIC *agents isolated*
1969 initial series	45	2
Comparison with yolk-sac method	44	2*
1970 series	62	4
Total nongonococcal urethritis cases	151	8
Reiter's syndrome	14	0

*Same two cases also + by yolk-sac method.

During the time of the study, urethral scrapings were obtained from fourteen patients with Reiter's syndrome but no TRIC agents were isolated.

Discussion

In our hands it was not possible to demonstrate that the McCoy cell method was more sensitive in isolating TRIC agents from urethral scrapings than the yolk-sac method. Although a lower overall isolation rate was obtained than in the previous study by the yolk-sac method in Vancouver, we believe that this was probably due to variables in case selection between the two studies performed 2 years apart. Such a view would be supported by the two identical isolations, when we compared the two methods on the same specimens. There was no doubt in our minds that the McCoy cell method was quicker, simpler, and economically preferable to the yolk-sac method. We had no problem in maintaining stock TRIC strains by repeated McCoy cell passage, and the method was reproducible when counts of inclusions were performed. The McCoy cell method, like the yolk-sac method, failed in our hands to demonstrate TRIC agents from a small group of patients with Reiter's syndrome.

Gordon and Quan (1971) have recently reported significantly higher isolation rates from patients with nongonococcal urethritis. The reasons for a discrepancy between our results and theirs are not apparent, even after personal discussions of the results. The majority of the patients in their recent studies were obtained from a V.D. Clinic in Washington, D.C., or Camp Lejeune, North Carolina, and their Washington patients were predominantly Negroes, in contrast to the Caucasian origin of our patients. The different isolation rates may be due to obscure technical deficiencies in our methods or alternatively there may be genuine differences in the incidence of TRIC agents between the two populations.

Summary

TRIC agents were isolated from eight urethral scrapings of 151 males with uncomplicated nongonococcal urethritis using the McCoy cell method of Gordon. Fourteen cases of Reiter's syndrome were also investigated with negative results. This method proved quicker and economically preferable, and its sensitivity was similar to, but not greater than, that of the yolk-sac technique.

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Isolement, par une méthode de culture sur cellules irradiés, de germes TRIC du type génital humain dans l'urétrite non gonococcique et la maladie de Reiter

SOMMAIRE

Des germes TRIC ont été trouvés, grâce à la méthode de Gordon sur cellules McCoy, dans huit grattages urétraux parmi 151 hommes atteints d'urétrite non gonococcique non compliquée. La recherche fut négative dans 14 cas de syndrome de Reiter. Cette méthode apparaît plus rapide et plus économique que la technique du sac vitellin; sa sensibilité est égale mais non supérieure.